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ELECTRICAL CONDUCTANCE OF AQUEOUS SUSPENSIONS OF MICROORGANISMS AND DETECTION OF LEAKAGE

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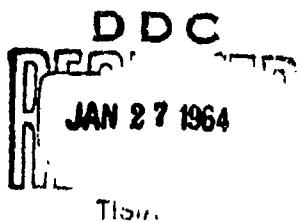
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Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 33

ELECTRICAL CONDUCTANCE OF AQUEOUS SUSPENSIONS
OF MICROORGANISMS AND DETECTION OF LEAKAGE

John S. Derr, Jr.

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The work reported here was performed under Project 4B11-02-068, Aerobiological Research, Task -02, "Physical and Chemical Studies of Aerosol Generation." The expenditure order number was 2072.

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ABSTRACT

The specific electrical conductivity of sterilized suspensions of
Pasteurella tularensis and Brucella suis was measured as a function of
temperature and compared with a standard sodium chloride solution for
naval application. A U.S. Navy Flooding Indicator was also tested with
these suspensions and compared with the standard solution. Results
indicated favorable application of the Flooding Indicator to detect
gross leakage of suspensions of these microorganisms under prescribed
standards.

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I. INTRODUCTION

At the request of MD Division, electrical conductance properties of two liquid suspensions of microorganisms, _Pasteurella_ _tularensis_ and _Brucella_ _suis_, have been measured. Because of the limited time allowed for this work, it was necessary to experiment in a clean area requiring sterilized slurries. These were provided by MR&AE Branch, Technical Evaluation Division. The specific lots, treatment, and certain physical properties determined before and after autoclaving are given in Appendix B as reported by Mr. Edward Golobic, Chief, Aerosol Evaluation Section, MR&AE Branch. There was no significant effect of autoclaving on the measured properties. However, if one of the effects of autoclaving is to release ions from the interior of the cell, conductivity of the slurry probably would be increased by increased free ion concentration and mobility of those ions freed from their membrane inclosure. We assume that release of cell contents to a free solution state would not appreciably alter the conductance properties. It still remains, however, to demonstrate that this is so. Consequently, the data in this report should be taken as somewhat tentative and useful for preliminary evaluation and planning, but not definitive in the formulation of standard operating procedures and instrumentation specifications.

Other considerations brought to light during the experimentation, which will be discussed below, also may be important enough to continue the study. Further, additional replication, improvement in temperature control, and additional calibration runs are perhaps desirable. This is a report only of work that provided data informally transmitted in response to a request by MD Division.

II. STATEMENT OF PROBLEM

The problem as posed was to determine whether a technique used by the Navy for detecting leakage of sea water would be applicable to the detection of leaks of biological suspensions. The technique consists of measuring current flow through a conducting net shorted by a quantity of liquid. The conducting net designated by the Bureau of Naval Weapons as "Indicator, Flooding, Mark 100 Mod 0,"[1] and the associated circuit used in the experiment and duplicating the required circuitry, is shown in Figure 1.

A Flooding Indicator was provided, together with requirements for operation: "3.4.3 Operation. The minimum current passing through the flooding indicator when shorted by one minim of salt water (35 points per 1000) shall be 1.5 milliamperes. Testing and acceptance shall be in accordance with 4.5.4.3;" "4.5.4.3 Operational Test. With the flooding indicator connected to the test circuit (Figure 1), the flooding indicator shall be shorted by one (1) minim of salt water (with a salinity of 35 parts per thousand). A current flow of less than 1.5 milliamperes shall be cause for rejection."[1]
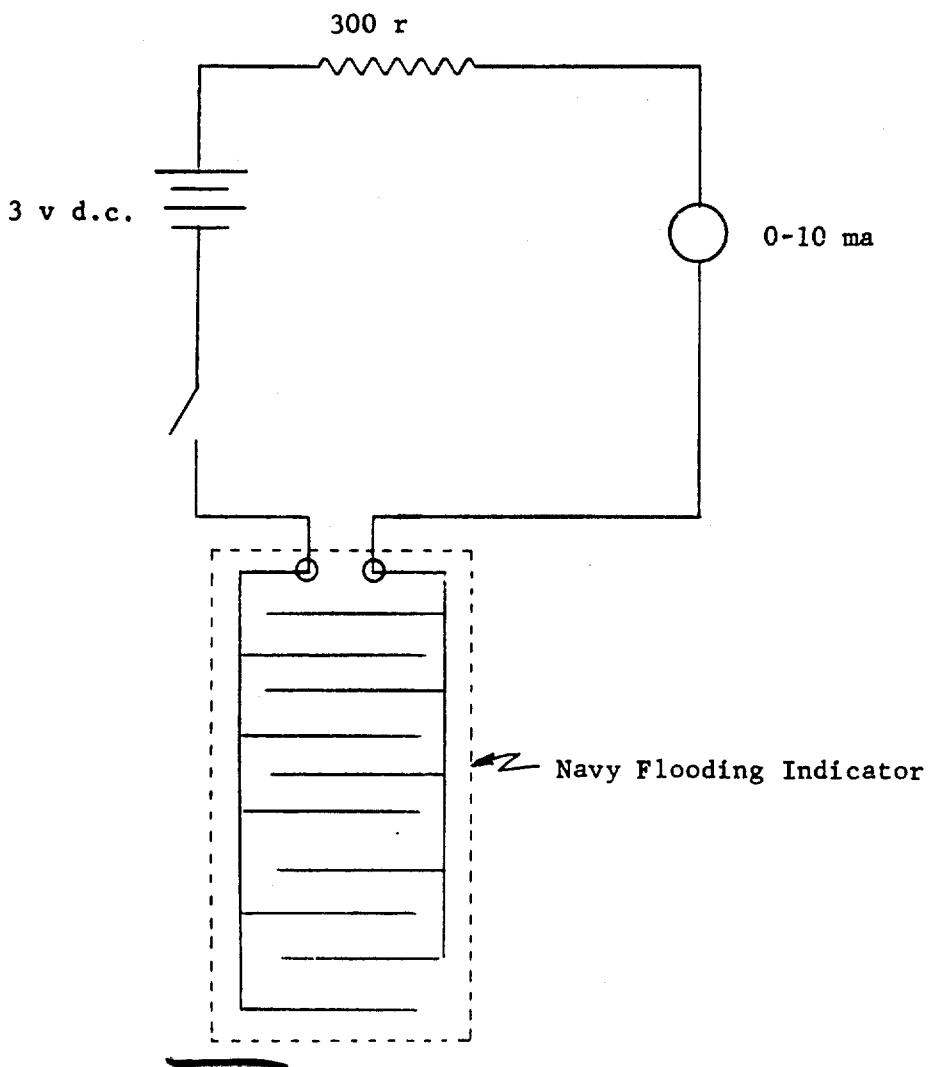
Figure 1. Flooding Indicator Test Circuit.

The term "salt water" in these requirements was interpreted to mean NaCl. It turned out that the measured specific conductivity of a 3.5 per cent water solution of NaCl (by weight NaCl to total weight of solution) at 20°C agreed within 0.6 per cent with a handbook value for sea water at the same temperature and "salinity," "salinity" being defined as approximately the "ratio of total solids to total sample of sea water."[2]*

Therefore, in order to determine the applicability of the Navy flooding indicator to the detection of suspension leakage it was decided to:

(a) Measure the specific electrical conductivity of sterile suspensions of <u>Pasteurella</u> <u>tularensis</u> and <u>Brucella</u> <u>suis</u> as a function of temperature.

(b) Measure the specific electrical conductivity of a 3.5 per cent weight NaCl to total weight of solution (35 parts per thousand) as a function of temperature.

(c) Perform conductance tests in accordance with specifications 4.5.4.3 with the 3.5 per cent NaCl, <u>Pasteurella</u> <u>tularensis</u>, and <u>Brucella</u> <u>suis</u>.

(d) Compare results with the two sets of measurements and draw conclusions.

III. MEASUREMENTS AND RESULTS

A. SPECIFIC ELECTRICAL CONDUCTIVITY

1. Calibration

Specific conductivity is defined[3]** by

$$R = \frac{L}{ka} \qquad (1)$$

where
R = resistance of solution
L = length of conductor
a = cross-sectional area (assumed uniform)
k = specific conductivity

Conductivity of a solution is a function of the nature of the electrolyte, the solvent, the concentration, and the temperature.[4] Measurement of specific conductivity of a solution requires a conductivity cell, consisting of two electrodes having a fixed area, <u>a</u>, and a distance apart, <u>L</u>.

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** Page 889.

Although subject to absolute calibration, the usual procedure is to experimentally determine L/a, the "cell constant," by measuring the resistance of a solution of known conductivity. The cell used in the measurements here was a so-called "dipping cell" (Figure 2) manufactured by Industrial Instruments, Inc., and having a nominal manufacturer's calibration of 0.10 cm^{-1}. Although ideally the calibration should be checked with KCl, so-called Kolrausch solutions, or subsequent determinations by the Parkers' method,[5] the cell constant was determined from an extrapolated conductivity value for 3.5 per cent NaCl given in Lange[6] and checked at various other concentrations with International Critical Table data. This value agreed with the nominal value

$$L/a = 0.104 \pm 0.001 \tag{2}$$

The electrical circuit used in determining the conductivity of the various solutions was provided by a standard impedance bridge (Type 650A Impedance Bridge with Type 650-P1 oscillator-amplifier manufactured by the General Radio Co.).

A driving voltage of 1000 cycles per second detected by earphones was used across the Wheatstone balancing circuit after the now-standard technique of Kohlrausch[3]* to eliminate polarization of the platinum electrodes and changes in resistance caused by liberated gas and bubble formation. A schematic view of the circuit is shown in Figure 3. No attempt was made to balance out the capacitive reactance of the cell, which in this case was small, sharp balance points being obtained. For high conductivities, the leads and electrode resistance of the cell may become significant. These were corrected in the following way:

$$K = \frac{\propto}{R} \tag{3}$$

where $\quad \propto$ = L/a = cell constant
R = solution resistance
k = specific conductivity

but $\quad R = R_m - r$

where $\quad r$ = lead and electrode resistance
R_m = measured resistance

Therefore
$$R = \frac{\propto}{R_m - r} \tag{4}$$

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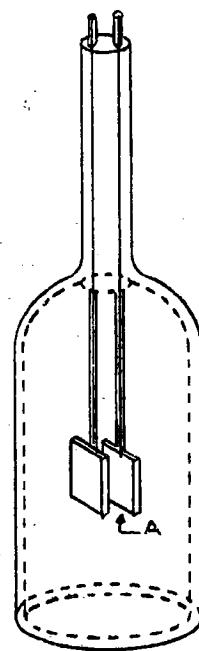
A   Platinum Electrode

Figure 2.   Conductance Cell.
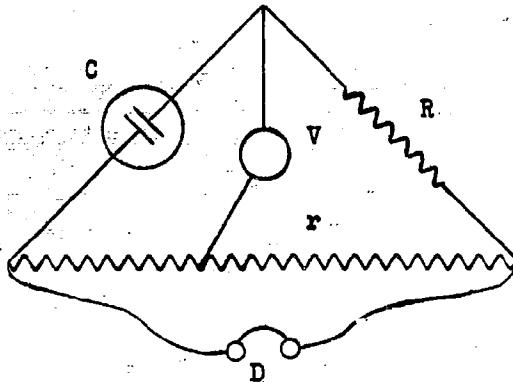
R   Fixed resistance   r   Variable resistance
V   Alternating 100° cps driving voltage   D   Earphone detector for null
C   Conductance cell

Figure 3.   Conductance Bridge Schematic Diagram.

The value of r was determined by measuring R_m for two solutions of known k. The value of $\propto$ also was determined from these two simultaneous equations

$$k_1 = \frac{\propto}{R_{m_1} \cdot r} = 0.046 \quad 3.5\% \text{ NaCl by weight at } 18°C \tag{5}$$

$$k_2 = \frac{\propto}{R_{m_2} \cdot r} = 0.251 \quad \begin{array}{l} \text{saturated NaCl at } 25°C \\ (361.5 \text{ gm in } 1000 \text{ ml } H_2O) \end{array}$$

From these measurements,

$$r = 0.074 \text{ ohm}$$

This represents a 14 per cent correction for solutions with conductivities of the order of 0.20 mho cm^{-1}.

To check the calibration, the specific conductivity of various concentrations of reagent-grade NaCl in distilled water was measured and compared with values given in the International Critical Tables (ICT).[7] These data are plotted in Figure 4. Measured values compared favorably with the table values. The test solutions were obtained by serial dilution of a saturated water solution of NaCl at 25°C. Handbook saturation data are expressed in terms of grams of NaCl in one liter of _water_. To convert to ICT concentrations expressed in grams of NaCl in one liter of solution, the specific molar density of NaCl in solution has to be calculated (Appendix A).

2. Specific Electrical Conductivity

The specific electrical conductivity of the suspensions and of the standard 3.5 per cent NaCl solution (made by dissolving 19.76 grams of NaCl in 545.40 grams of water) was measured as a function of temperature by the procedure described above. The cell temperature was not controlled but was merely measured immediately before and after a conductivity measurement. Data were plotted at the mean of the two readings. Typical temperature rises during a reading were $\pm 2°C$ at 5°C level and $\pm 0.5°C$ at the 20°C level. Results are plotted in Figure 5. The average deviation for a given conductivity measurement was of the order of one per cent. Also shown for comparison in Figure 5 is the conductivity of sea water at a concentration of 35 parts per thousand (3.5 per cent by weight) taken from the American Institute of Physics Handbook[2]* as well as the value for the conductivity of 3.5 per cent NaCl extrapolated from Lange's Handbook.[8]**

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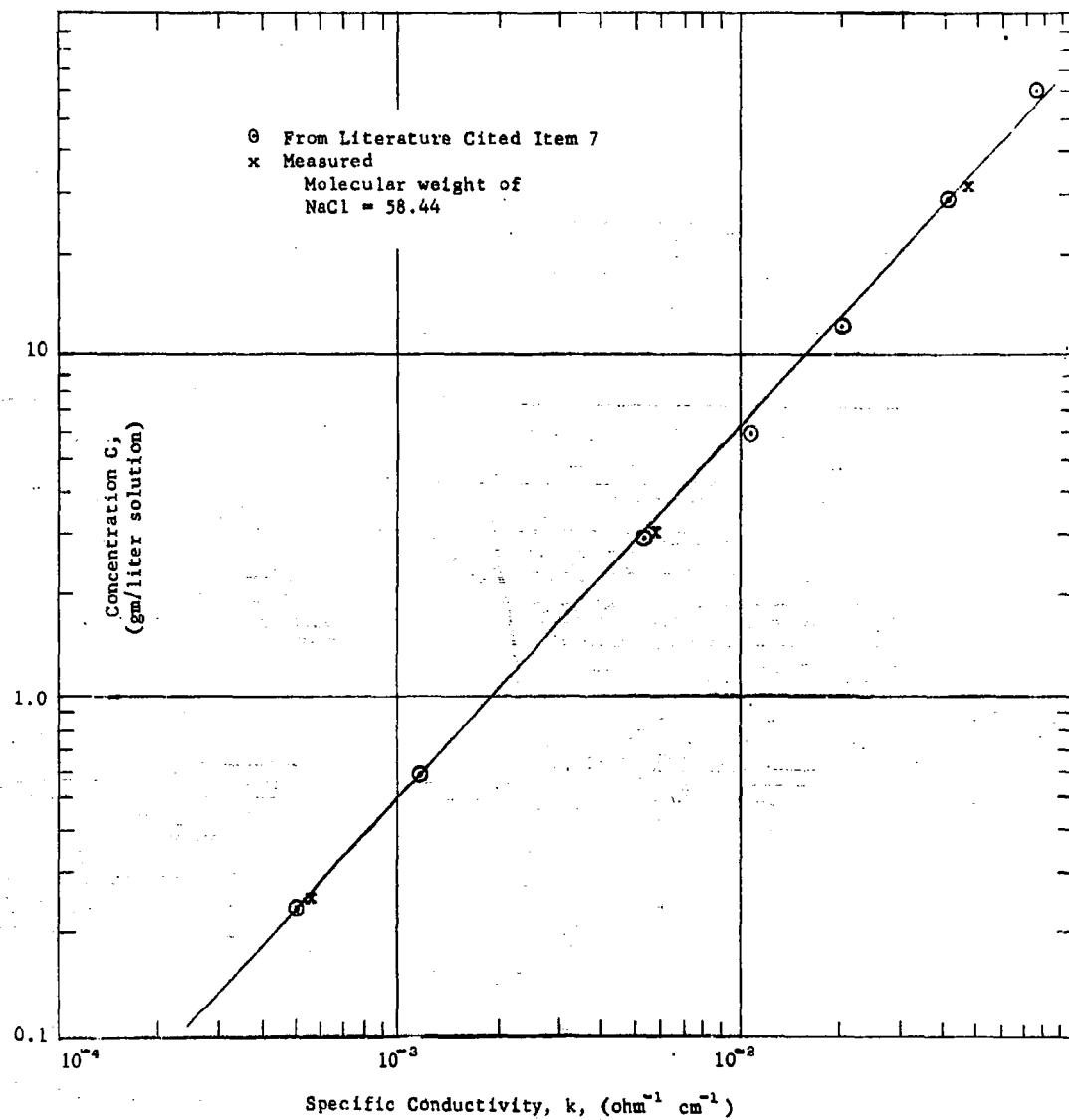
Figure 4. Specific Conductivity of NaCl at 25°C as a Function of Concentration.
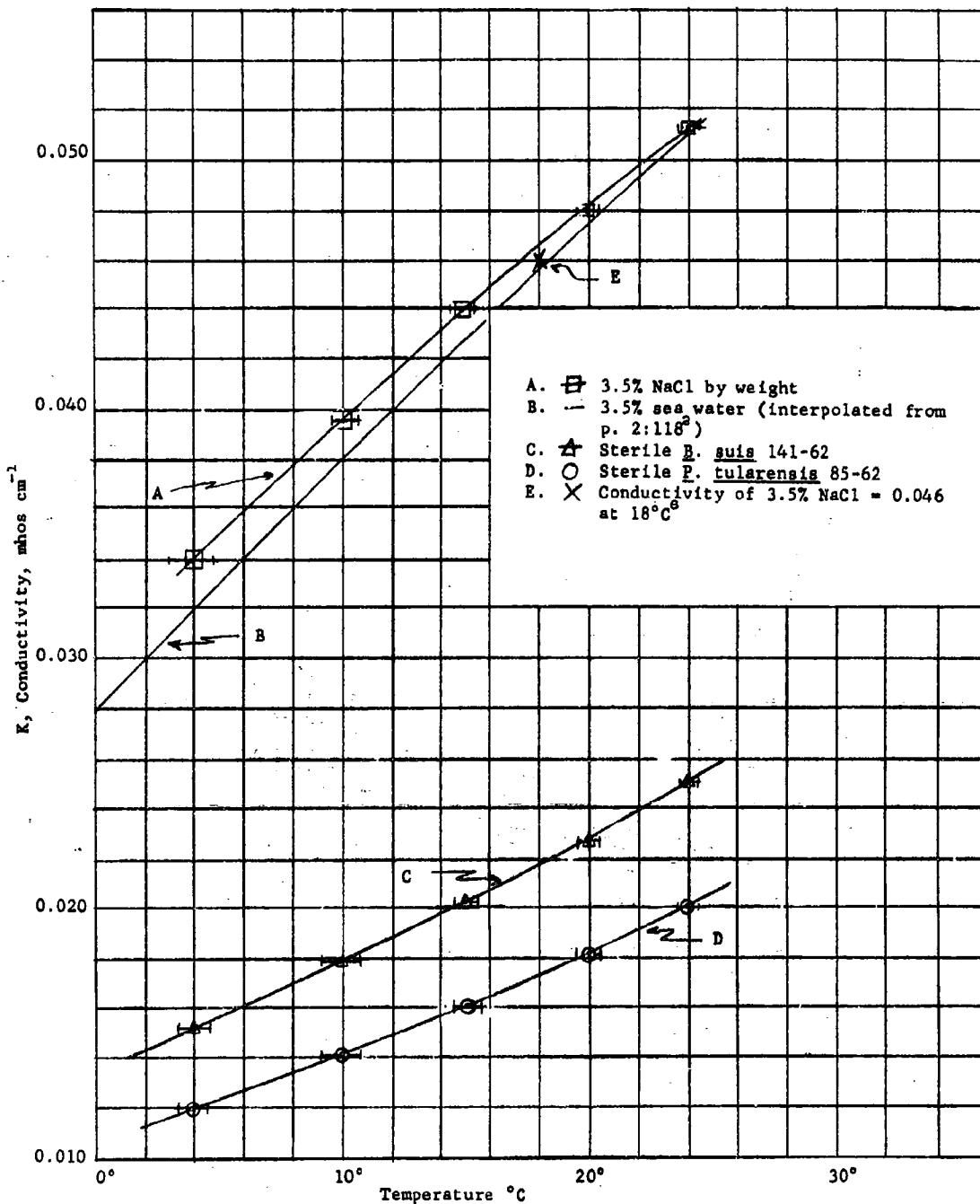
Figure 5. Conductivity vs Temperature of Killed Biological Suspensions and Salt Water.

The procedure used to sterilize the suspensions and their various physical properties before and after sterilization are given in Appendix B. As mentioned earlier, there are no significant differences in physical properties resulting from the sterilizing process. The electrical conductivity, however, could have been modified by release to solution of freely diffusible ions, which, if anything, would then tend to increase the conductivity. This remains to be resolved. Results are summarized in Table I. Values are taken from the curves of Figure 5.

TABLE I. SPECIFIC ELECTRICAL CONDUCTIVITY k (mho cm^{-1}) OF SUSPENSIONS AND 3.5 PER CENT SODIUM CHLORIDE SOLUTION

Liquid	Temperature, °C				
	5	10	15	20	25
B. suis	0.015_6	0.017_9	0.020_2	0.022_7	0.025_2
P. tularensis	0.012_2	0.014_2	0.016_2	0.018_2	0.020_4
NaCl, 3.5%	0.035_0	0.039_6	0.043_8	0.047_9	0.051_8

In general, the specific electrical conductivity of the B. suis is about half that of the standard salt solution and of P. tularensis about one-third of the standard. Further, all solutions have a monotonically increasing function of k with temperature, with the standard solution slightly concave downward and the agents conversely concave upward toward increasing conductivity.

B. NAVY FLOODING INDICATOR TESTS

1. Circuit Resistance of the Indicator

The Flooding Indicator was tested for circuit resistance, using the General Radio Impedance Bridge, across A - B and C - D as shown in Figure 6 and as required by the acceptance specification 4.5.4.2. Values obtained were A - B, 0.047 ohm, C - D, 0.052 ohm.

Open-circuit resistance across A - C was greater than 20 megohms as determined by VTVM. Acceptance specifications require A - B and C - D resistance to be less than 0.10 ohm and A - C resistance, using 500 volts d.c., to be greater than 100 megohms.
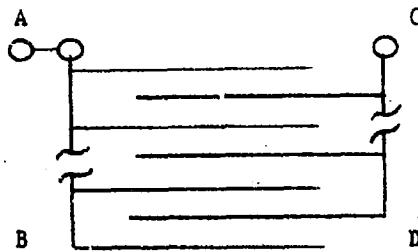
Figure 6. Indicator Schematic Diagram.

2. Flooding Indicator Conductance Test

With the Flooding Indicator circuit open (Figure 3), a 0.06-ml drop of the test liquid was placed at random on the Indicator. The drop was formed by superimposing liquid from two micro-burets delivering 0.05 and 0.01 ml respectively. (Specifications require "one-minim" drops or 0.062 ml). Next, the circuit was closed and current was measured with a milliammeter, with qualitative observations in its time dependence. Invariably, an initial transient pulse was observed that rapidly settled down to a steady state. Further, there was some evidence for long-term changes in the steady-state phase. A quantitative study of time dependence of current flow in the Indicator circuit with a one-minim drop as the conducting path was not made, and performance was based on quasi-steady-state current values. Results and observations for the two suspensions and NaCl are given in Table II. Average results of the Flooding Indicator Conductance tests are given in Table IIa with average deviations.

3. Direct Current Resistance of Liquid Drops on Flooding Indicator

As a further test of the electrical characteristics of the Flooding Indicator shorted by drops of the various test liquids, the d.c. resistance of the Indicator-drop-shorted circuit was measured with the Impedance Bridge. Because of the low Indicator resistance, this measurement essentially was that of the drop. A potential of 18 volts d.c. was used in the drop-Indicator arm of the balancing circuit, and electrolysis effects were therefore different from those in the Flooding Indicator test circuit utilizing three volts d.c. Generally speaking, the resistance was also a long-term function of time, indicating polarization and bubble resistance effects. Results are indicated in Table III. The run number corresponds to the same drop cited in Table II. Flooding Indicator tests were performed first.

TABLE II. NAVY FLOODING INDICATOR CURRENTS FOR TEST LIQUIDS[a]

Liquid	Run No.	Temperature, °C	Steady Current, ma	Observations
P. tularensis 85-62	1	26.0	1.8	4 strips covered
	2	26.0	1.0	Drop foamy after current flow
	3	26.0	2.3	Bubbles in drop from buret
	4	26.0	1.7	One large bubble from buret
	5	26.0	2.4	No buret bubble
	6	26.4	2.4	4 strips covered
	7	26.4	2.6	

Small bubbles of gas were generated in each run, with bubbles moving in drop from ± to -. Run 6 current went from 2.8 ma initial pulse to 2.4 ma in 10 seconds.

Liquid	Run No.	Temperature, °C	Steady Current, ma	Observations
B. suis 141-62	1	26.4	2.8	Drop touched 4 strips
	2	26.4	3.6	
	3	26.4	2.4	Drop touched 5 strips, buret bubble
	4	26.4	3.2	No buret bubbles, 4-strip contact

Gas formation bubbles occurred in each drop. Run 2 current went from 4 ma to 3.6 ma in 4 seconds.

Liquid	Run No.	Temperature, °C	Steady Current, ma	Observations
NaCl, 3.5%	1	25.6	1.5	3 strips covered
	2	25.6	0.8	3 strips covered
	3	25.6	1.8	Initial surge to 3 ma
	4	25.6	2.4	
	5	25.6	1.5	Initial surge to 3.8 ma, steady after 4 sec.

a. Slight hydrolysis and copper electrode dissolution.

TABLE IIa. AVERAGE FLOODING INDICATOR CURRENT VALUES

Liquid	Current, ma	Temperature, °C
P. tularensis	2.0 ± 0.4	26.0
B. suis	3.0 ± 0.4	26.4
NaCl	1.6 ± 0.4	25.6

TABLE III. IMPEDANCE BRIDGE RESISTANCE OF DROPS ON FLOODING INDICATOR

Liquid	Run No.	Temperature, °C	Steady State d.c. Resistance, r, ohms	Observation
P. tularensis 85-62	6	26.4	1200	After 1 min many fine electrolysis bubbles
	7	26.4	760	Steady state after 45-sec fluctuation between 500 r and 1.5 Kr. White milky appearance. Navy Indicator current after this measurement = 3.8 ma.
B. suis 141-62	1	26.4	580	Vigorous bubble formation
	4	26.4	660	Foamy white bubbles in center of drop
NaCl, 3.5%	3	25.6	3300	Steady state
	5	25.6	3000	Drop initially circular 7 mmd. Became square during measurement. Resistance increased from 420 r to 3 Kr after 2 min in circuit

TABLE IIIa. AVERAGE VALUES OF DROP RESISTANCE

Liquid	Average d.c. Resistance, r, ohms
P. tularensis	980
B. suis	620
NaCl	3150

As can be seen from a comparison of data in Table I and Table IIa, current through the drops of the several liquids cannot be correlated per se with specific electrical conductivity. Remember that specific electrical conductivity was measured with a conductance cell in an a.c. circuit, preventing polarization and extraneous resistance effects due to bubble formation. Further, as indicated by Equation (1), the geometry of the conducting path further controls total drop resistance.

The resistance of drops also can be determined from the Flooding Indicator Circuit (using 3 volts d.c.) data and compared with the Impedance Bridge data, Table IIIa, utilizing 18 volts bridge voltage.

Resistance by the Flooding Indicator is given by

$$R = \frac{E}{I} - r \qquad\qquad (6)$$

where
- I = measured current
- E = EMF in circuit (3 volts)
- R = drop resistance
- r = 300 ohms circuit resistance

These Flooding Indicator resistances are compared with d.c. bridge measurements in Table IV for the same drops. Differences must be attributable to differences in polarization and bubble resistance effects resulting from electrolysis as a function of voltage.

TABLE IV. FLOODING INDICATOR RESISTANCE VS IMPEDANCE BRIDGE
MEASURED RESISTANCE OF DROPS

| Liquid	Run No.	Calculated Resistance by Flooding Indicator,[a] r, ohms		Measured Resistance by Impedance Bridge,[b] r, ohms	
F. tularensis	6	950		1200	
			900 ± 50		980 ± 220
	7	850		760	
B. suis	1	770		580	
			705 ± 65		620 ± 40
	4	640		660	
NaCl 3.5%	3	1370		3300	
			1535 ± 200		3150 ± 150
		1700		3000	

a. 3 volts d.c.
b. 18 volts d.c.

Note that the biological suspensions give about the same resistance values for each measurement, whereas NaCl shows a significant difference, with the 18-volt resistance higher by a factor of two. This indicates that polarization and bubble resistance effects are more important with NaCl than with the suspensions, since they are more sensitive with applied voltage. This contributes to an explanation as to why d.c. current flow in the Flooding Indicator circuit is smaller through the salt drop than through the suspensions despite the fact that specific electrical conductivity is higher by a factor of about two. Another explanation for this discrepancy in current flow correlation with specific electrical conductivity can, however, be proposed. This involves

(a) The geometric term in Equation (2), L/a, the ratio of conducting path length to conducting area and

(b) The resistance net actually formed by the drop.

As indicated in Table II above, the number of copper conducting strips touched by the drops on the Flooding Indicator was different for the suspensions and NaCl, four strips and three strips respectively. Hence, equivalent circuits for the drops can be given as shown below.

Biological Suspensions   NaCl
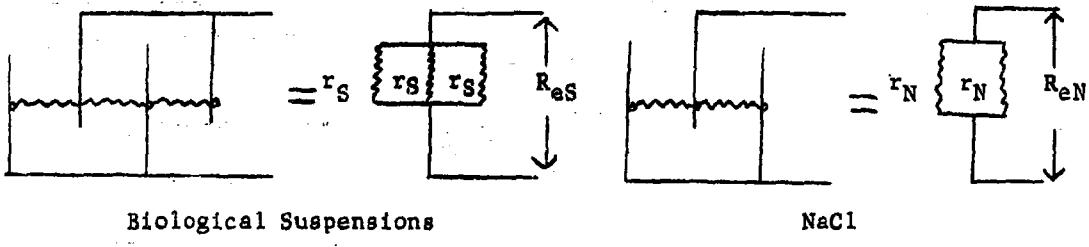
Since the drops can be considered quasi-parallelepiped, the effective drop resistance in the Flooding Indicator can be related to the actual drop resistance by the following equations.

$$\frac{1}{R_{eS}} = \frac{3}{r_S} \qquad \text{Suspensions}$$

$$\frac{1}{R_{eN}} = \frac{2}{r_N} \qquad \text{NaCl}$$

where R_{eS} = effective resistance of drop of suspension

               R_{eN} = effective resistance of drop of NaCl

               r_S = resistance of portion of drop of suspension between two adjacent Flooding Indicator strips

               r_N = resistance of portion of drop of NaCl between two adjacent Flooding Indicator strips

But

$$r_S = \frac{R_S}{3} \text{ and } r_N = \frac{R_N}{2} \tag{8}$$

where R_S = actual resistance of suspension

               R_N = actual resistance of NaCl drop

$$R_{eS} = \frac{R_S}{9} \text{ and } R_{eN} = \frac{R_N}{4} \tag{9}$$

R_S and R_N can be computed from Flooding Indicator 3-volt d.c. readings. These are shown in Table V.

TABLE V. EFFECTIVE DROP RESISTANCE AND ACTUAL
DROP RESISTANCE

Liquid	$R_{e(SN)}$, r, ohms	$R_{(SN)}$, r, ohms
P. tularensis	900	8100
B. suis	705	6345
NaCl	1535	6140

      Using Equation (1) to describe the resistance of a drop and equating it to values of R_N in Table V, and further using measured values of specific electrical conductivity given in Table I taken at 25°C, a geometry factor, L/a, can be computed for each suspension.

$$R = \frac{L}{ka} = R_N$$

These values are shown in Table VI. Note that the value of L/a for NaCl computed using the conductance cell determination of k does not correspond with observations on its spreading relative to biological suspension.

TABLE VI. GEOMETRY FACTOR FOR TEST LIQUIDS

Liquid	k, mho cm^{-1}	R_N, ohms	L/a, cm^{-1}	σ, dynes cm^{-1}
P. tularensis	0.0204	8100	162	51.8
B. suis	0.025	6345	160	55.0
NaCl	0.053	6140	320	74.0

The geometry factor in Equation (1) for a drop on a flat surface is related to the spreading of the drop. Since all drops have identical volumes, this spreading is then a function only of the wetting ability of the liquid, which again is related to the interfacial surface tension of the liquid, i.e., spreading (area per unit volume) is inversely proportional to surface tension. For a flat drop, approaching a parallelepiped and resting on flat electrodes at opposite edges of the drop,

let

t = drop thickness
D = drop length or width
f = factor of proportionality (f = 1 for a continuous conducting medium)
L = conducting path length
a = conducting area normal to flow

then

$L = D$
$a = fDt$

and

$$\frac{L}{a} = \frac{1}{ft}$$

Average values of t were measured for drops of each liquid on the Flooding Indicator. These are given in Table VII.

TABLE VII. AVERAGE DROP THICKNESS

Liquid	Volume, cm^3	Area, cm^2	Average Thickness, t, cm	Surface Tension, dynes cm^{-1}
P. tularensis	0.060	0.64	0.093	51.8
B. suis	0.060	0.64	0.093	55.0
NaCl	0.060	0.36	0.167	74.0^8*

* Page 2192.

Hence, f for each liquid can be computed using these values of t and values of L/a from Table VI, in turn calculated from measured specific conductivities. These values are shown in Table VIII.

TABLE VIII. COMPUTED VALUES OF CONDUCTION AREA FACTOR, f

Liquid	L/a, cm^{-1}	t, cm	f
P. tularensis	162	0.093	0.066
B. suis	160	0.093	0.067
NaCl	320	0.167	0.018

The physical significance of f is not known. It represents in part the proportion of the total area for ionic flow, and in this sense could reflect a noncontinuous cross section resulting from bubbles, or a selective path resulting from a nonuniform electric field in the drop. On the other hand, since f was calculated from specific conductivity data obtained using the bulk liquid alternating current bridge technique, it could reflect a difference in specific electrical conductivity due to polarization of the liquid in the d.c. field associated with the Flooding Indicator circuit.

Assuming a uniform conducting path and parallel electric lines of force within the drops, i.e., letting f = 1, an "apparent" specific electrical conductivity for a drop of liquid in the Flooding Indicator can be computed and compared with the conductance bridge measurement. This comparison is shown in Table IX, using L/a apparent = 1/t and Equation (1).

TABLE IX. APPARENT DROP CONDUCTIVITY

Liquid	L/a, cm^{-1} (Apparent)	k, mho cm^{-1} (Conductance Bridge)	k, mho cm^{-1} (Apparent)	k Apparent / k Conductance Bridge
P. tularensis	10.7	0.020	1.32×10^{-3}	0.066
B. suis	10.7	0.025	1.67×10^{-3}	0.067
NaCl	6.0	0.053	0.97×10^{-3}	0.018

The ratio of "apparent" specific electrical conductivity to conductance-bridge-measured specific electrical conductivity can be interpreted to reflect polarization effects, greater for NaCl than for the biological suspension. This ratio is of course identical to f as computed above using the conducting path geometry interpretation.

IV. CONCLUSIONS AND SUMMARY

The specific electrical conductivity of two sterilized biological suspensions, P. tularensis and B. suis, was measured as a function of temperature and compared with values obtained for a 3.5 per cent (by weight) NaCl water solution. The specific electrical conductivities of the suspensions were lower by about a factor of two than for the standard NaCl solution in the temperature range studied.

The Navy Flooding Indicator was tested, using a Navy SOP for determining level of performance. In each case the killed suspensions performed better than the standard NaCl solution in order of decreasing acceptability B. suis, P. tularensis, 3.5 per cent NaCl.

Reasons for the apparent discrepancy between the two conclusions above have been presented and calculations made in support of the hypothesis. It seems that three factors may be involved to varying degrees in effecting higher Flooding Indicator currents with the suspensions compared with the salt solution:

(a) Smaller polarization effects on the part of the suspensions.

(b) Decreased geometry factor, L/a, in the total drop resistance of the suspensions compared with the salt solution.

(c) As a result of increased spreading of the suspension drops, due to decreased surface tension, decreased effective drop resistance through formation of a lower resistance parallel network.

The various measurements were performed with suspensions killed by autoclaving. A comparison of physical properties (see Appendix B) of killed vs non-killed suspensions shows some possible slight effect in pH in the case of B. suis and in viscosity and surface tension of P. tularensis. We surmise that these differences are such that the performance of the live suspensions with the Flooding Indicator will not be appreciably different from that of the killed suspensions. Possibly, the decreased surface tension of the killed P. tularensis will decrease resistance slightly in comparison with the live suspension, other things being equal. On the other hand, a slight decrease in measured pH is indicated that may off-set this effect.

Pending conclusive measurements on live suspensions, it can be assumed that one-minim drops of B. suis and P. tularensis will perform as well as one-minim drops of 3.5 per cent (by weight) NaCl water solution with the Navy Flooding Indicator.

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APPENDIX A

CONVERSION OF CONCENTRATION UNITS

With a table of specific gravity of aqueous sodium chloride solutions and per cent NaCl by weight of solution,[8]* weight of water per liter of solution could be obtained, and from this the weight and solution volume of NaCl:

Per Cent NaCl (by weight)	Specific Gravity	NaCl, gm/liter of Solution at 20°C
6	1.0413	62.48
24	1.1804	283.3

a. For 6% (by wt) NaCl   1041.3 grams in one liter of solution
                                        62.5 grams NaCl in one liter
                               978.8 grams H_2O in one liter

     (1000.0 - 978.8) ml = 21.2 ml NaCl displacement
     Solution density NaCl = 62.5/21.2 = 2.94 gm/ml

b. For 24% (by wt) NaCl   1180.4 grams in one liter of solution
                                          283.3 grams NaCl in one liter
                               897.1 grams H_2O in one liter

     102.9 ml displaced by NaCl

     Solution density NaCl $= \dfrac{283.3}{102.9} = 2.75$ grams per milliliter

This compares with the crystal density of NaCl of 2.16. Hence, corcentrstion of NaCl expressed in grams of NaCl per liter of solution can be calculated.

At 25°C, saturated NaCl solution contains 361.5 grams in one liter of water.[8]** Hence:

     Volume displaced 361.5/2.75 = 130.5 ml
     Volume of solution = 1130.5 ml, containing 361.5 gm NaCl

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* Page 2070.
** Page 1701.

Concentration expressed in grams of NaCl per liter of solution:

$361.5/1130.5 = 319.7$ gm/liter

Concentration expressed in a per cent by weight of NaCl to total weight:

per cent weight $= 361.5/1361.5 = 26.5\%$

Calculated specific gravity of saturated NaCl solution at 25°C:

$$\frac{361.5 + 1000}{1000 + 130.5} = 1.204 \text{ gm/cm}^3$$

APPENDIX B

PROPERTIES OF BIOLOGICAL SUSPENSIONS

Table I shows the physical properties of viable and sterilized B. suis and P. tularensis suspensions.*

TABLE I. PROPERTIES OF P. TULARENSIS AND B. SUIS SUSPENSIONS

(Temperature of Measurements = 30°C)

Suspension	Treatment	Surface Tension, dynes/cm	pH	Specific Gravity	Viscosity, centipoise	Per Cent Dry Weight	Cell Count, 10^9/ml
B. suis	Viable	57.6	6.7	1.018	1.174	4.0	37
(141-62)	Killed	55.0	7.0	1.021	1.229	4.1	
P. tularensis	Viable	55.1	6.6	1.024	1.580	5.2	315
(85-62)	Killed	51.8	6.4	1.024	2.177	5.4	

The sterilization procedure for the sterilized suspensions used in the study consisted of autoclaving with steam for 30 minutes at 255°F and 21 psig.

* Provided by Mr. E.T. Colobic, Chief, Aerosol Evaluation Section, MR&AE Branch, Technical Evaluation Division.